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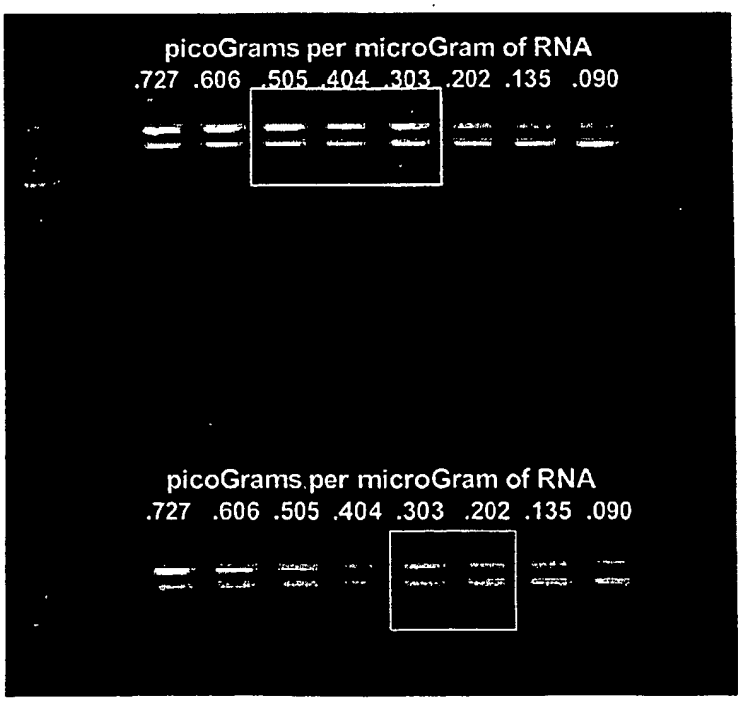
(54) Title: TREATMENT OF NEURODEGENERATIVE DISEASE THROUGH INTRACRANIAL DELIVERY OF SIRNA



(57) Abstract: The present invention provides devices, small interfering RNA, and methods for treating a neurodegenerative disorder comprising the steps of surgically implanting a catheter so that a discharge portion of the catheter lies adjacent to a predetermined infusion site in a brain, and discharging through the discharge portion of the catheter a predetermined dosage of at least one substance capable of inhibiting production of at least one neurodegenerative protein. The present invention also provides valuable small interfering RNA vectors, and methods for treating neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, Spinocerebellar Ataxia Type 1, Type 2, Type 3, and/or dentatorubral-pallidoluysian atrophy.

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TREATMENT OF NEURODEGENERATIVE DISEASE THROUGH INTRACRANIAL DELIVERY OF SIRNA

FIELD OF INVENTION

This invention relates to devices, systems, and methods for treating neurodegenerative disorders by brain infusion of small interfering RNA or vectors containing the DNA encoding for small interfering RNA.

BACKGROUND OF THE INVENTION

This invention provides novel devices, systems, and methods for delivering small interfering RNA to targeted sites in the brain to inhibit or arrest the development and progression of neurodegenerative disorders. For several neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, Huntington's disease, Spinocerebellar Ataxia Type 1, Type 2, and Type 3, and dentatorubral pallidoluysian atrophy (DRLPA), proteins involved in the overall pathogenic progression of the disease have been identified. There is currently no cure for these neurodegenerative diseases. These diseases are progressively debilitating and most are ultimately fatal.

Further problematic of these neurodegenerative diseases (especially Alzheimer's disease and Parkinson's disease) is that their prevalence continues to increase, thus creating a serious public health problem. Recent studies have pointed to alpha-synuclein (Parkinson's disease), beta- amyloid-cleaving enzyme 1 (BACE1 (including variants thereof, e.g. variants A, B, C, and D)) (Alzheimer's disease), huntingtin (Huntington's disease), and ataxin 1 (Spinocerebellar Ataxia Type 1) as major factors in the pathogenesis of each of these diseases, respectively.

The neurodegenerative process in Parkinson's disease and Alzheimer's disease is characterized by extensive loss of selected neuronal cell populations accompanied by synaptic injury and astrogliosis. Pathological hallmarks of Alzheimer's disease include formation of amyloid plaques, neurofibrillary tangles and neuropil thread formation; pathological hallmarks of Parkinson's diseases include the formation of intraneuronal inclusions called Lewy bodies and the loss of dopaminergic neurons in the substantia

nigra. Although the mechanisms triggering cell dysfunction and death are unclear, the prevailing view is that neurodegeneration results from toxic effects subsequent to the accumulation of specific neuronal cell proteins, such as alpha-synuclein (Parkinson's disease) and amyloid precursor protein (APP) (Alzheimer's disease – processed into beta-amyloid by BACE1 (including variants thereof, e.g. variants A, B, C, and D)).

Alpha-synuclein has been implicated in Parkinson's disease because it is abundantly found in Lewy Bodies, its overexpression in transgenic mice leads to Parkinson's disease-like pathology, and mutations within this molecule are associated with familial Parkinson's disease. Alpha-synuclein, which belongs to a larger family of molecules including β and γ-synuclein, is a 140 amino acid non-amyloid synaptic protein which is a precursor of the 35 amino acid non-amyloid component protein found in amyloid plaques.

Alzheimer's disease is a progressive degenerative disorder of the brain characterized by mental deterioration, memory loss, confusion, and disorientation. Among the cellular mechanisms contributing to this pathology are two types of fibrillar protein deposits in the brain: intracellular neurofibrillary tangles composed of polymerized tau protein, and abundant extracellular fibrils comprised largely of β-amyloid. Beta-amyloid, also known as Aβ, arises from the proteolytic processing of the amyloid precursor protein (APP) at the the β- and γ- secretase cleavage sites giving rise to the cellular toxicity and amyloid-forming capacity of the two major forms of Aβ ($A\beta_{40}$ and $A\beta_{42}$). Thus, preventing APP processing into plaque-producing forms of amyloid may critically influence the formation and progression of the disease making BACE1 (including variants thereof, e.g. variants A, B, C, and D) a clinical target for inhibiting or arresting this disease. Similar reports suggest presenilins are candidate targets for redirecting aberrant processing.

Huntington's disease is a fatal, hereditary neurodegenerative disorder characterized by involuntary "ballistic" movements, depression, and dementia. The cause has been established to be a mutation in a single gene consisting of an excessively long series of C, A, G, C, A, G, ... C, A, G, nucleotides in the DNA. The CAG repeat is in the region of the gene that codes for the protein the gene produces. Thus, the resulting huntingtin

protein is also "expanded," containing an excessively long region made of the amino acid glutamine, for which "CAG" encodes. Shortly after this mutation was pinpointed as the cause of Huntington's disease, similar CAG repeat expansions in other genes were sought and found to be the cause of numerous other fatal, hereditary neurodegenerative diseases. The list of these so-called "polyglutamine" diseases now includes at least eleven more, including: spinocerebellar ataxia type 1, type 2, and type 3, spinobulbar muscular atrophy (SBMA or Kennedy's disease) and dentatorubral-pallidoluysian atropy (DRPLA). Although the particular gene containing the expanded CAG repeat is different in each disease, it is the production of an expanded polyglutamine protein in the brain that causes each one. Symptoms typically emerge in early to middle-aged adulthood, with death ensuing 10 to 15 years later. No effective treatments for these fatal diseases currently exist.

There is considerable evidence suggesting that shutting off production of the abnormal protein in neurons will be therapeutic in polyglutamine diseases. The cause of these diseases is known to be the gain of a new function by the mutant protein, not the loss of the protein's original function. Mice harboring the human, expanded transgene for spinocerebellar ataxia type 1 (SCA1) become severely ataxic in young adulthood (Clark, H., *et al.*, *Journal of Neuroscience* 17: 7385-7395 (1997)), but mice in which the corresponding mouse gene has been knocked out do not suffer ataxia or display other major abnormalities (Matilla, A., *et al.*, *Journal of Neuroscience* 18: 5508-5516 (1998)). Transgenic mice for SCA1 in which the abnormal ataxin1 protein is produced but has been genetically engineered to be incapable of entering the cell's nucleus do not develop ataxia (Klement, I., *et al.*, *Cell* 95: 41-53 (1998)). Finally, a transgenic mouse model of Huntington's disease has been made in which the mutant human transgene has been engineered in a way that it can be artificially "turned off" by administering tetracycline (Normally, in mice and humans, administration of this antibiotic would have no effect on the disease). After these mice have begun to develop symptoms, shutting off production of the abnormal protein production by chronic administration of tetracyclin leads to an improvement in their behavior (Yamamoto, A., *et al.*, *Cell* 101: 57-66 (2000)). This suggests that reducing expression of the abnormal huntingtin protein in humans might not

only prevent Huntington's disease from progressing in newly diagnosed patients, but may improve the quality of life of patients already suffering from its symptoms.

Various groups have been recently studying the effectiveness of siRNAs. Caplen , *et al. (Human Molecular Genetics,* 11(2): 175-184 (2002)) assessed a variety of different double stranded RNAs for their ability to inhibit cell expression of mRNA transcripts of the human androgen receptor gene containing different CAG repeats. Their work found only gene –specific inhibition occurred where flanking sequences to the CAG repeats were present in the double stranded RNAs. They were also able to show that constructed double stranded RNAs were able to rescue induced caspase-3 activation. Xia, Haibin, et al. (*Nature Biotechnology,* 20: 1006-1010 (2002)) tested the inhibition of polyglutamine (CAG) expression of engineered neural PC12 clonal cell lines that express a fused polyglutamine-fluorescent protein using constructed recombinant adenovirus expressing siRNAs targeting the mRNA encoding green fluorescent protein.

The design and use of small interfering RNA complementary to mRNA targets that produce particular proteins is a recent tool employed by molecular biologist to prevent translation of specific mRNAs. Other tools used by molecular biologist interfere with translation involve cleavage of the mRNA sequences using ribozymes against therapeutic targets for Alzheimer's disease (see WO01/16312A2) and Parkinson's disease (see WO99/50300A1 and WO01/60794A2). However, none of the above aforementioned patents disclose methods for the specifically localized delivery of small interfering RNA vectors to targeted cells of the brain in a manner capable of local treatment of neurodegenerative diseases. The above patents do not disclose use of delivery devices or any method of delivery or infusion of small interfering RNA vectors to the brain. For example, the above patents do not disclose or suggest a method of delivery or infusion of small interfering RNA vectors to the brain by an intracranial delivery device.

Further, the foregoing prior art does not disclose any technique for infusing into the brain small interfering RNA vectors, nor does the prior art disclose whether small interfering RNA vectors, upon infusion into the brain, are capable of entering neurons and producing the desired small interfering RNA, which is then capable of reducing

production of at least one protein involved in the pathogenesis of neurodegenerative disorders.

The prior art describes direct systemic delivery of ribozymes. This approach for treatment of neurodegenerative disorders would appear neither possible nor desirable. First, interefering RNAs are distinctly different than ribozymes. Second, small RNA molecules delivered systemically will not persist in vivo long enough to reach the desired target, nor are they likely to cross the blood-brain barrier. Further, the approach taken by the prior art may be impractical because of the large quantity of small interfering RNA that might have to be administered by this method to achieve an effective quantity in the brain. Even when the blood-brain barrier is temporarily opened, the vast majority of oligonucleotide delivered via the bloodstream may be lost to other organ systems in the body, especially the liver.

U.S. Patent Nos. 5,735,814 and 6,042,579 disclose the use of drug infusion for the treatment of Huntington's disease, but the drugs specifically identified in these patents pertain to agents capable of altering the level of excitation of neurons, and do not specifically identify agents intended to enter the cell and alter protein production within cells.

The present invention solves prior problems existing in the prior art relating to systemic delivery of nucleic acids by directly delivering small interfering RNA in the form of DNA encoding the small interfering RNA to target cells of the brain using viral vectors. Directed delivery of the small interfering RNA vectors to the affected region of the brain infusion overcomes previous obstacles related to delivery. Further, use of viral vectors allows for efficient entry into the targeted cells and for efficient short and long term production of the small interfering RNA agents by having the cells' machinery direct the production of the small interfering RNA themselves. Finally, the present invention provides a unique targeting and selectivity profile by customizing the active small interfering RNA agents to specific sites in the mRNA coding sequences for the offending proteins.

SUMMARY OF THE INVENTION

The present invention provides devices, systems, methods for delivering small interfering RNA for the treatment of neurodegenerative disorders.

A first objective of the described therapies is to deliver specifically tailored small interfering RNA as therapeutic agents for treatment of Parkinson's disease. Specifically tailored small interfering RNA for Parkinson's disease target the mRNA for the alpha-synuclein protein in order to reduce the amount of alpha-synuclein protein produced in neurological cells. In a related embodiment the present invention provides devices that specifically access the substantia nigra for delivery of anti-alpha-synuclein small interfering RNA.

A second objective of the described therapies is to deliver specifically tailored small interfering RNA as therapeutic agents for treatment of Alzheimer's disease. Specifically tailored small interfering RNA for Alzheimer's disease target the mRNA for BACE1 (including variants thereof, e.g. variants A, B, C, and D) in order to reduce the amount of BACE1 (including variants thereof, e.g. variants A, B, C, and D) protein produced in neurological cells and thereby interfere with the production of beta-amyloid. In a related embodiment the present invention provides devices that specifically access the nucleus basalis of Meynart and the cerebral cortex for delivery of anti-BACE1 (including variants thereof, e.g. variants A, B, C, and D) small interfering RNA.

A third objective of the described therapies is to deliver specifically tailored small interfering RNA as therapeutic agents for treatment of Huntington's disease. Specifically tailored small interfering RNA for Huntington's disease target the mRNA for huntingtin protein to reduce the amount of huntingtin protein produced in neurological cells. In a related embodiment the present invention provides devices that specifically access the caudate nucleus and putamen (collectively known as the striatum) for delivery of anti-huntingtin small interfering RNA.

A fourth objective of the described therapies is to deliver specifically tailored small interfering RNA as therapeutic agents for treatment of Spinocerebellar Ataxia Type 1 (SCA1). Specifically tailored small interfering RNA for Spinocerebellar Ataxia Type 1

target the mRNA for ataxin1 protein to reduce the amount of ataxin1 protein produced in neurological cells. In a related embodiment the present invention provides devices that specifically access the dentate nucleus, eboliform nucleus, globus nucleus, and fastigial nucleus of the cerebellum, (collectively known as the deep cerebellar nuclei), for delivery of anti-ataxin-1 small interfering RNA.

A fifth objective of the described therapies is to deliver specifically tailored small interfering RNA as therapeutic agents for treatment of Spinocerebellar Ataxia Type 3 (SCA3), also known as Machado-Joseph's Disease. Specifically tailored small interfering RNA for Spinocerebellar Ataxia Type 3 target the mRNA for ataxin3 protein to reduce the amount of ataxin3 protein produced in neurological cells. In a related embodiment the present invention provides devices that specifically access the dentate nucleus, eboliform nucleus, globus nucleus, and fastigial nucleus of the cerebellum, (collectively known as the deep cerebellar nuclei), the subthalamic region, and the substantia nigra for delivery of anti-ataxin-3-small interfering RNA.

A sixth objective of the described therapies is to deliver specifically tailored small interfering RNA as therapeutic agents for treatment of dentatorubral-pallidoluysian atrophy (DRPLA). Specifically tailored small interfering RNA for DRPLA target the mRNA for atrophin-1 protein to reduce the amount of atrophin-1 protein produced in neurological cells. In a related embodiment the present invention provides devices that specifically access the dentate nucleus, eboliform nucleus, globus nucleus, and fastigial nucleus of the cerebellum, (collectively known as the deep cerebellar nuclei), the globus pallidus, and the red nucleus for delivery of anti-DRPLA small interfering RNA.

The present invention provides a delivery system for a small interfering RNA vector therapy for neurodegenerative diseases that permits targeted delivery of small interfering RNA or vectors containing DNA encoding for small interfering RNA (small interfering RNA vectors) to targeted sites in the brain for brief durations of time or over an extended period of care for the patient.

In a main embodiment of the present invention, small interfering RNA vectors are infused into targeted sites of the brain wherein the small interfering RNA vectors are taken up by neurons and transported to the nucleus of targeted cells. The small interfering RNA

vectors are then transcribed into RNA by the host cellular machinery to produce small interfering RNA that prevent production of the targeted neurodegenerative protein.

The present invention also provides methods of using neurosurgical devices to deliver therapeutic small interfering RNA vectors to selected regions of the brain. In particular, the present invention provides methods that use surgically implanted catheters for singular, repeated, or chronic delivery of small interfering RNA vectors to the brain. The small interfering RNA vectors introduced into the affected cells have the necessary DNA sequences for transcription of the required small interfering RNA by the cells, including a promoter sequence, the small interfering RNA sequence, and optionally flanking regions allowing defined ends of the therapeutic small interfering RNA to be produced, and optionally a polyadenylation signal sequence.

DESCRIPTION OF THE FIGURES

Figure 1 shows the assay (using a quantitative RT-PCR method known to those practiced in the art) of the ataxin1 mRNA obtained from HEK293H cells that have been transfected with plasmid containing an anti-ataxin1 ribozyme (top lanes in Figure 1) or with siRNA against ataxin1 (bottom lanes of Figure 1).

Figure 2 shows the assay (using the same quantitative RT-PCR method known to those practiced in the art) of the ataxin-1 mRNA obtained from HEK293H cells that have been transfected with anti-ataxin-1 small interfering RNA (bottom lanes) compared to the mRNA obtained from HEK293H cells that have been transfected with a control siRNA that targets the mRNA for glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Figure 3 shows the construction of the adeno-associated virus expression vector pAAV-siRNA.

Figure 4 illustrates an investigational device (by Medtronic, Inc. of Minneapolis, MN Model 8506), which can be implanted subcutaneously on the cranium, and provides an access port through which therapeutic agents may be delivered to the brain.

Figure 5 illustrates an investigational device (by Medtronic, Inc. of Minneapolis, MN - schematic of Model 8506), which can be implanted subcutaneously on the cranium, and provides an access port through which therapeutic agents may be delivered to the brain.

Figure 6 illustrates the relation of various neurodegenerative diseases described herein, and the location of treatment with small interfering RNA vectors directed to their intended targeted gene product.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention solves two problems in the prior art at the same time: (1) the problem of how to treat neurodegenerative diseases caused by the production in neurons of a protein that has pathogenic properties and (2) the problem of delivery of therapeutic small interfering RNA to affected neurons.

In order to better understand the present invention, a list of terms and the scope of understanding of those terms is provided below.

Terminology

By "alpha-synuclein, BACE1 (including variants thereof, e.g. variants A, B, C, and D), huntingtin, ataxin-1, ataxin-3, and/or atrophin-1 proteins" is meant, a protein or a mutant protein derivative thereof, comprising the amino-acid sequence expressed and/or encoded by alpha-synuclein (Parkinson's disease), and beta-site APP-cleaving enzyme (BACE1 (including variants thereof, e.g. variants A, B, C, and D)) (Alzheimer's disease), huntingtin (Huntington's disease), and ataxin-1 (Spinocerebellar Ataxia Type 1), ataxin-3 (Spinocerebellar Ataxia Type 3 or Machado-Joseph's Disease), and/or dentatorubral-pallidoluysian atrophy (DRPLA) genes and/or the human genomic DNA respectively.

As used herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism. The cell may be present in an organism which may be a human but is preferably of mammalian origin, e.g., such as humans, cows, sheep, apes, monkeys, swine, dogs, cats, and the like. However, several steps of producing small

interfering RNA may require use of prokaryotic cells (e.g., bacterial cell) or eukaryotic cell (e.g., mammalian cell) and thereby are also included within the term "cell".

By "complementarity" it is meant that a molecule comprised of one or more nucleic acids (DNA or RNA) can form hydrogen bond(s) with another molecule comprised of one or more nucleic acids by either traditional Watson-Crick pairing or other non- traditional types.

By "equivalent" DNA to alpha-synuclein, BACE1 (including variants thereof, e.g. variants A, B, C, and D), huntingtin, ataxin-1, ataxin-3, and/or atrophin-1 it is meant to include those naturally occurring DNA molecules having homology (partial or complete) to DNA encoding for alpha-synuclein, BACE1 (including variants thereof, e.g. variants A, B, C, and D), huntingtin, ataxin-1, ataxin-3 and/or atrophin-1 proteins or encoding for proteins with similar function as alpha-synuclein, BACE1 (including variants thereof, e.g. variants A, B, C, and D), huntingtin, ataxin-1, ataxin-3 and/or atrophin-1 in various organisms, including human, rodent, primate, rabbit, pig, and microorganisms. The equivalent DNA sequence also includes regions such as the 5'-untranslated region, the 3'-untranslated region, introns, intron-exon junctions, small interfering RNA targeted site and the like, optionally incorporated into the DNA of infective viruses, such as adeno-associated virus (AAV).

The term "functional equivalent" refers to any derivative that is functionally similar to the reference sequence or protein. In particular the term "functional equivalent" includes derivatives in which the nucleotide bases(s) have been added, deleted, or replaced without a significant adverse effect on biological function.

By "gene" it is meant a region of DNA that controls the production of RNA. In context of producing functional small interfering RNA, this definition includes the necessary DNA sequence information encompassing the DNA sequences encoding the small interfering RNA, noncoding regulatory sequence and any included introns. The present definition does not exclude the possibility that additional genes encoding proteins may function in association or in tandem with the genes encoding small interfering RNA.

The term "vector" is commonly known in the art and defines a plasmid DNA, phage DNA, viral DNA and the like, which can serve as a DNA vehicle into which DNA

of the present invention can be inserted, and from which RNA can be transcribed. The term "vectors" refers to any of these nucleic acid and/or viral-based techniques used to deliver a desired nucleic acid. Numerous types of vectors exist and are well known in the art.

The term "expression" defines the process by which a gene is transcribed into RNA (transcription); the RNA may be further processed into the mature small interfering RNA.

The terminology "expression vector" defines a vector or vehicle as described above but designed to enable the expression of an inserted sequence following transformation into a host. The cloned gene (inserted sequence) is usually placed under the control of control element sequences such as promoter sequences. The placing of a cloned gene under such control sequences is often referred to as being operably linked to control elements or sequences.

"Promoter" refers to a DNA regulatory region capable of binding directly or indirectly to RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of the present invention, the promoter is bound at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter will be found a transcription initiation site (conveniently defined by mapping with S1 nuclease), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CCAT" boxes. Prokaryotic promoters contain -10 and -35 consensus sequences, which serve to initiate transcription.

By "homology" it is meant that the nucleotide sequence of two or more nucleic acid molecules is partially or completely identical.

By "highly conserved sequence region" it is meant that a nucleotide sequence of one or more regions in a target gene does not vary significantly from one generation to the other or from one biological system to the other.

By the term "inhibit" or "inhibitory" it is meant that the activity of the target genes or level of mRNAs or equivalent RNAs encoding target genes is reduced below that

observed in the absence of the provided small interfering RNA. Preferably the inhibition is at least 10% less, 25% less, 50% less, or 75% less, 85% less, or 95%less than in the absence of the small interfering RNA.

By "inhibited expression" it is meant that the reduction of alpha-synuclein, BACE1 (including variants thereof, e.g. variants A, B, C, and D), huntingtin, ataxin-1, ataxin-3 and/or atrophin-1 mRNA levels and thus reduction in the level of the respective protein to relieve, to some extent, the symptoms of the disease or condition.

By "RNA" is meant ribonucleic acid, a molecule consisting of ribonucleotides connected via a phosphate-ribose(sugar) backbone. By "ribonucleotide" is meant guanine, cytosine, uracil, or adenine or some a nucleotide with a hydroxyl group at the 2' position of a β-D- ribo-furanose moiety. As is well known in the art, the genetic code uses thymidine as a base in DNA sequences and uracil in RNA. One skilled in the art knows how to replace thymidine with uracil in a nucleic acid sequence to convert a DNA sequence into RNA, or vice versa.

By "patient" is meant an organism, which is a donor or recipient of explanted cells or the cells themselves. "Patient" also refers to an organism to which the nucleic acid molecules of the invention can be administered. Preferably, a patient is a mammal or mammalian cells, e.g., such as humans, cows, sheep, apes, monkeys, swine, dogs, cats, and the like, or cells of these animals used for transplantation. More preferably, a patient is a human or human cells.

The term "synuclein" may refer to alpha-synuclein (especially human or mouse) or beta-synuclein (especially human or mouse). The full nucleotide sequence encoding human alpha-synuclein is available under Accession No AF163864 (SEQ ID:7). Two variants of the human alpha-synuclein sequence are available under Accession No NM000345 (SEQ ID:14) and Accession No NM_007308 (SEQ ID:23). The mouse alpha-synuclein is available under Accession No. AF163865 (SEQ ID:10).

The term "BACE1" may refer to beta-site amyloid precursor protein cleaving enzyme type 1 (especially human or mouse). Several variants of BACE1 have been sequenced, including variants A, B, C, and D. In some scientific literature, BACE1 is also known as ASP2 and Memapsin2. The full nucleotide sequences encoding human BACE1,

and variants related thereto, are available under Accession No. NM_138971 (SEQ ID:20), Accession No. NM_138972 (SEQ ID:19), Accession No. NM_138973 (SEQ ID:21), and Accession No. NM_012104 (SEQ ID:18). The sequence for a mouse homolog is available under accession number NM_011792 (SEQ ID:22).

The term "huntingtin" may refer to the protein product encoded by the Huntington's Disease gene (IT-15) (especially human or mouse). The full nucleotide sequence encoding human IT-15 is available under Accession No AH003045 (SEQ ID:9). The mouse sequence is available under Accession No. U24233 (SEQ ID:12).

The term "ataxin-1" may refer to the protein product encoded by the Spinocerebellar Ataxia Type 1 gene (especially human or mouse). The full nucleotide sequence encoding human SCA1 is available under Accession No NM_000332 (SEQ ID:15). The mouse sca1 is available under Accession No. NM_009124 (SEQ ID:13).

The term "ataxin-3" may refer to the protein product encoded by the Spinocerebellar Ataxia Type 3 gene (especially human or mouse). The full nucleotide sequence encoding human SCA3 is available under Accession No NM_004993 (splice variant 1) (SEQ ID:16), and NM_030660 (splice variant 2) (SEQ ID:17). (The sequence for a mouse homolog is not yet available).

The term "atrophin-1" may refer to the protein product encoded by the dentatorubral-pallidolysian atrophy (DRPLA) gene (especially human or mouse). The full nucleotide sequence encoding human DRPLA is available under Accession No XM_032588 (SEQ ID:8). The mouse sequence is available under Accession No. XM_132846 (SEQ ID:11).

The term "modification" includes derivatives substantially similar to the reference sequence or protein.

By "nucleic acid molecule" as used herein is meant a molecule having nucleotides. The nucleic acid can be single, double, or multiple stranded and may comprise modified or unmodified nucleotides or non-nucleotides or various mixtures and combinations thereof. An example of a nucleic acid molecule according to the invention is a gene which encodes for a small interfering RNA, even though it does not necessarily have its more common meaning for encoding for the production of protein.

By "small interfering RNA" is meant a nucleic acid molecule which has complementarity in a substrate binding region to a specified gene target, and which acts to specifically guide enzymes in the host cell to cleave the target RNA. That is, the small interfering RNA by virtue of the specificity of its sequence and its homology to the RNA target, is able to cause cleavage of the RNA strand and thereby inactivate a target RNA molecule because it is no longer able to be transcribed. These complementary regions allow sufficient hybridization of the small interfering RNA to the target RNA and thus permit cleavage. One hundred percent complementarity often necessary for biological activity and therefore is preferred, but complementarity as low as 90% may also be useful in this invention. The specific small interfering RNA described in the present application are not meant to be limiting and those skilled in the art will recognize that all that is important in a small interfering RNA of this invention is that it have a specific substrate binding site which is complementary to one or more of the target nucleic acid regions.

Small interfering RNAs are double stranded RNA agents that have complementary to (i.e., able to base-pair with) a portion of the target RNA (generally messenger RNA). Generally, such complementarity is 100%, but can be less if desired, such as 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. For example, 19 bases out of 21 bases may be base-paired. In some instances, where selection between various allelic variants is desired, 100% complementary to the target gene is required in order to effectively discern the target sequence from the other allelic sequence. When selecting between allelic targets, choice of length is also an important factor because it is the other factor involved in the percent complementary and the ability to differentiate between allelic differences.

XXXX

The small interfering RNA sequence needs to be of sufficient length to bring the small interfering RNA and target RNA together through complementary base-pairing interactions. The small interfering RNA of the invention may be of varying lengths. The length of the small interfering RNA is preferably greater than or equal to ten nucleotides and of sufficient length to stably interact with the target RNA; specifically 15-30 nucleotides; more specifically any integer between 15 and 30 nucleotides, such as 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, and 30. By "sufficient length" is meant

an oligonucleotide of greater than or equal to 15 nucleotides that is of a length great enough to provide the intended function under the expected condition. By "stably interact" is meant interaction of the small interfering RNA with target nucleic acid (e.g., by forming hydrogen bonds with complementary nucleotides in the target under physiological conditions).

By "comprising" is meant including, but not limited to, whatever follows the word "comprising". Thus, use of the term "comprising" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present.

By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present.

By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

The present invention provides the means and tools for treating polyglutamine diseases (such as Huntington's disease and spinocerebellar ataxia type 1), Parkinson's disease, and Alzheimer's disease by intracranial delivery of vectors encoding small interfering RNAs designed to silence the expression of disease-causing or disease-worsening proteins, delivered through one or more implanted intraparenchymal catheters. In particular, the invention is (1) a method to treat Huntington's disease by the intracranial delivery of a vector encoding a small interfering RNA designed to silence expression of huntingtin protein; (2) a method to treat spinocerebellar ataxia type 1 by the intracranial delivery of a vector encoding a small interfering RNA designed to silence expression of ataxin1 protein; (3) a method to treat Parkinson's disease by the intracranial delivery of a vector encoding a small interfering RNA designed to silence expression of alpha-synuclein protein, and (4) a method to treat Alzheimer's disease by the intracranial delivery of a

vector encoding a small interfering RNA designed to silence expression of beta-amyloid cleaving enzyme 1 (BACE1).

As previously indicated, the small interfering RNA (or siRNA) described herein, is a segment of double strandedRNA that is from 15 to 30 nucleotides in length. It is used to trigger a cellular reaction known as RNA interference. In RNA interference, double-stranded RNA is digested by an intracellular enzyme known as Dicer, producing siRNA duplexes. The siRNA duplexes bind to another intracellular enzyme complex which is thereby activated to target whatever mRNA molecules are homologous (or complementary) to the siRNA sequence. The activated enzyme complex cleaves the targeted mRNA, destroying it and preventing it from being used to direct the synthesis of its corresponding protein product. By means that are not yet fully understood, the RNA interference process appears to be self-amplifying. Recent evidence suggests that RNA interference is an ancient, innate mechanism for not only defense against viral infection (many viruses introduce foreign RNA into cells) but also gene regulation at very fundamental levels. RNA interference has been found to occur in plants, insects, lower animals, and mammals, and has been found to be dramatically more effective than other gene silencing technologies, such as antisense or ribozymes. Used as a biotechnology, siRNA involves introducing into cells (or causing cells to produce) short, double-stranded molecules of RNA similar to those that would be produced by the Dicer enzyme from an invading double-stranded RNA virus. The artificially-triggered RNA interference process then continues from that point.

To deliver a small interfering RNA to a patient's brain, the preferred method will be to introduce the DNA encoding for the siRNA, rather than the siRNA molecules themselves, into the cells of the brain. The DNA sequence encoding for the particular therapeutic siRNA can be specified upon knowing (a) the sequence for a small and accessible portion of the target mRNA (available in public human genome databases), and (b) well-known scientific rules for how to specify DNA that will result in production of a corresponding RNA sequence when the DNA is transcribed by cells. The DNA sequence, once specified, can be constructed in the laboratory from synthetic molecules ordered from

a laboratory supplier, and inserted using standard molecular biology methods into one of several alternative "vectors" for delivery of DNA to cells. Once delivered into the neurons of the patient's brain, those neurons will themselves produce the RNA that becomes the therapeutic siRNA, by transcribing the inserted DNA into RNA. The result will be that the cells themselves produce the siRNA that will silence the targeted gene. The result will be a reduction of the amount of the targeted protein produced by the cell.

Small interfering RNA and Small interfering RNA Vectors

In accordance with the present invention, small interfering RNA against specific mRNAs produced in the affected cells prevent the production of the disease related proteins in neurons. In accordance with the present invention is the use of specifically tailored vectors designed to deliver small interfering RNA to targeted cells. The success of the designed small interfering RNA is predicated on their successful delivery to the targeted cells of the brain to treat the neurodegenerative diseases.

Small interfering RNA have been shown to be capable of targeting specific mRNA molecules in human cells. Small interfering RNA vectors can be constructed to transfect human cells and produce small interfering RNA that cause the cleavage of the target RNA and thereby interrupt production of the encoded protein.

A small interfering RNA vector of the present invention will prevent production of the pathogenic protein by suppressing production of the neuropathogenic protein itself or by suppressing production of a protein involved in the production or processing of the neuropathogenic protein. Repeated administration of the therapeutic agent to the patient may be required to accomplish the change in a large enough number of neurons to improve the patient's quality of life. Within an individual neuron, however, the change is longstanding enough to provide a therapeutic benefit. The desperate situation of many patients suffering from neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, or Spinocerebellar Ataxia Type 1 provides a strong likelihood that the benefit from the therapy will outweigh the risks of the therapy delivery and administration. While it may be possible to accomplish some reduction in the production of neuropathogenic proteins with other therapeutic agents and routes of

administration, development of successful therapies involving direct in vivo transfection of neurons may provide the best approach based on delivery of small interfering RNA vectors to targeted cells.

The preferred vector for delivery of foreign DNA to neurons in the brain is adeno-associated virus (AAV), such as recombinant adeno-associated virus serotype 2 or recombinant adeno-associated virus serotype 5. Alternatively, other viral vectors, such as herpes simplex virus, may be used for delivery of foreign DNA to central nervous system neurons. It is also possible that non-viral vectors, such as plasmid DNA delivered alone or complexed with liposomal compounds or polyethyleneamine, may be used to deliver foreign DNA to neurons in the brain.

It is important to note that the anti-ataxin-1 small interfering RNA illustrated here, as well as the other small interfering RNAs for treating neurodegenerative disorders, are just but some examples of the embodiment of the invention. Experimentation using neurosurgical methods with animals, known to those practiced in neuroscience, can be used to identify the candidate small interfering RNAs. The target cleavage site and small interfering RNA identified by these empirical methods will be the one that will lead to the greatest therapeutic effect when administered to patients with the subject neurodegenerative disease.

In reference to the nucleic molecules of the present invention, the small interfering RNA are targeted to complementary sequences in the mRNA sequence coding for the production of the target protein, either within the actual protein coding sequence, or in the 5' untranslated region or the 3' untranslated region. After hybridization, the host enzymes are capable of cleavage of the mRNA sequence. Perfect or a very high degree of complementarity is needed for the small interfering RNA to be effective. A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. However, it should be noted that

single mismatches, or base-substitutions, within the siRNA sequence can substantially reduce the gene silencing activity of a small interfering RNA.

The small interfering RNA that target the specified sites in alpha-synuclein, BACE1 (including variants thereof, e.g. variants A, B, C, and D), huntingtin, ataxin-1, ataxin-3 and/or atrophin-1 RNAs represent a novel therapeutic approach to treat Parkinson's disease, Alzheimer's disease, Huntington's disease, Spinocerebellar 1, Spinocerebellar Ataxia Type 3, and/or dentatorubral-pallidoluysian atrophy in a cell or tissue.

In preferred embodiments of the present invention, a small interfering RNA is 15 to 30 nucleotides in length. In particular embodiments, the nucleic acid molecule is 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. In preferred embodiments the length of the siRNA sequence can be between 19-30 base pairs, and more preferably between 21 and 25 base pairs, and more preferably between 21 and 23 basepairs.

In a preferred embodiment, the invention provides a method for producing a class of nucleic acid-based gene inhibiting agents that exhibit a high degree of specificity for the RNA of a desired target. For example, the small interfering RNA is preferably targeted to a highly conserved sequence region of target RNAs encoding alpha-synuclein, BACE1 (including variants thereof, e.g. variants A, B, C, and D), huntingtin, ataxin-1, ataxin-3 and/or atrophin-1 RNA such that specific treatment of a disease or condition can be provided with either one or several nucleic acid molecules of the invention. Further, generally, interfering RNA sequences are selected by identifying regions in the target sequence that begin with a pair of adenine bases (AA)(see Examples). SiRNAs can be constructed in vitro or in vivo using appropriate transcription enzymes or expression vectors.

SiRNAs can be constructed in vitro using DNA oligonucleotides. These oligonucletides can be constructed to include an 8 base sequence complementary to the 5' end of the T7 promoter primer included in the Silencer siRNA (Ambion Construction Kit 1620). Each gene specific oligonucleotide is annealed to a supplied T7 promoter primer, and a fill-in reaction with Klenow fragment generates a full-length DNA template for

transcription into RNA. Two in vitro transcribed RNAs (one the antisense to the other) are generated by in vitro transcription reactions then hybridized to each other to make double-stranded RNA. The double-stranded RNA product is treated with DNase (to remove the DNA transcription templates) and RNase (to polish the ends of the double-stranded RNA), and column purified to provide the siRNA that can be delivered and tested in cells.

Construction of siRNA vectors that express siRNAs within mammalian cells typically use an RNA polymerase III promoter to drive expression of a short hairpin RNA that mimics the structure of an siRNA. The insert that encodes this hairpin is designed to have two inverted repeats separated by a short spacer sequence. One inverted repeat is complementary to the mRNA to which the siRNA is targeted. A string of thymidines added to the 3' end serves as a pol III transcription termination site. Once inside the cell, the vector constitutively expresses the hairpin RNA. The hairpin RNA is processed into an siRNA which induces silencing of the expression of the target gene, which is called RNA interference (RNAi)..

In most siRNA expression vectors described to date, one of three different RNA polymerase III (pol III) promoters is used to drive the expression of a small hairpin siRNA (1-5). These promoters include the well-characterized human and mouse U6 promoters and the human H1 promoter. RNA pol III was chosen to drive siRNA expression because it expresses relatively large amounts of small RNAs in mammalian cells and it terminates transcription upon incorporating a string of 3–6 uridines.

The constructed nucleic acid molecules can be delivered exogenously to specific tissue or cellular targets as required. Alternatively, the nucleic acid molecules (e.g., small interfering RNA) can be expressed from DNA plasmid , DNA viral vectors, and/or RNA retroviral vectors that are delivered to specific cells.

The delivered small nuclear RNA sequences delivered to the targeted cells or tissues are nucleic acid-based inhibitors of alpha-synuclein, BACE1 (including variants thereof, e.g. variants A, B, C, and D), huntingtin, ataxin-1, ataxin-3 and/or atrophin-1 expression (e.g. translational inhibitors) are useful for the prevention of the

neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, Huntington's disease, Spinocerebellar Ataxia Type 1, Spinocerebellar Ataxia Type 3, and DRPLA and any other condition related to the level of alpha-synuclein, BACE1 (including variants thereof, e.g. variants A, B, C, and D), huntingtin, ataxin-1, ataxin-3 and/or atrophin-1 in a cell or tissue, and any other diseases or conditions that are related to the levels of alpha-synuclein, beta-amyloid, huntingtin, ataxin-1, ataxin-3 or atrophin-1 in a cell or tissue.

The nucleic acid-based inhibitors of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, packaged within viral vectors, or otherwise delivered to target cells or tissues. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the nucleic acid inhibitors comprise sequences which are a sufficient length and/or stably interact with their complementary substrate sequences identified in SEQ ID NOS: 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23. Examples of such small interfering RNA also are shown in SEQ IDS NOS: 1, 2, 3, 4, for SEQ IDS relating to Ataxin1.

In another aspect, the invention provides mammalian cells containing one or more nucleic acid molecules and/or expression vectors of this invention. The one or more nucleic acid molecules may independently be targeted to the same or different sites.

In another aspect of the invention, small interfering RNA molecules that interact with target RNA molecules and inhibit alpha-synuclein, BACE1 (including variants thereof, e.g. variants A, B, C, and D), huntingtin, ataxin-1, ataxin-3 and/or atrophin-1 RNA activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Small interfering RNA expressed from viral vectors could be constructed based on, but not limited to, the vector sequences of adeno-associated virus, retrovirus, or adenovirus. Preferably, the recombinant vectors capable of expressing the small interfering RNA are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of small interfering RNA. Such vectors might be

repeatedly administered as necessary. Once expressed, the small interfering RNA bind to the target RNA and through use of the host machinery inhibit its expression and thereby its function. Delivery of small interfering RNA expressing vectors, or the small interfering RNA themselves, is by use of intracranial access devices.

The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to treat diseases or conditions discussed above. For example, to treat a disease or condition associated with alpha-synuclein (Parkinson's Disease), and beta-site APP-cleaving enzyme (Alzheimer's Disease), huntingtin (Huntington's Disease), and Ataxin 1 (Spinocerebellar Ataxia) , the patient may be treated, or other appropriate cells may be treated, as is evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment.

In a further embodiment, the described small interfering RNA can be used in combination with other known treatments to treat conditions or diseases discussed above.

In another preferred embodiment, the invention provides nucleic acid- based inhibitors (e.g., small interfering RNA) and methods for their use to downregulate or inhibit the expression of RNA (e.g., alpha-synuclein, BACE1 (including variants thereof, e.g. variants A, B, C, and D), huntingtin, ataxin-1, ataxin-3 and/or atrophin-1) coding for proteins involved in the progression and/or maintenance of Parkinson's disease, Alzheimer's disease, Huntington's disease, Spinocerebellar Ataxia Type 1, Spinocerebellar Ataxia Type 3, and dentatorubral-pallidoluysian atrophy.

The present invention also provides nucleic acid molecules that can be expressed within cells from known eukaryotic promoters (e.g., Izant and Weintraub, 1985, Science, -229, 345; McGarry and Lindquist, 1986, Proc. Natl. Acad. Sci., USA 83, 399; Scanlon et al., 1991, Proc. Natl. Acad. Sci. USA, 88, 10591-5; Kashani- Sabet et al., 1992, Antisense Res. Dev., 2, 3-15; Dropulic et al., 1992, J Virol., 66, 1432- 41; Weerasinghe et al., 1991, J Virol., 65, 5531-4; Ojwang et al., 1992, Proc. Natl. Acad. Sci. USA, 89, 10802-6; Chen et al., 1992, Nucleic Acids Res., 20, 4581-9; Sarver et al., 1990 Science, 247, 1222-1225; Thompson et al., 1995, Nucleic Acids Res., 23, 2259; Good et al., 1997, Gene Therapy, 4, 45; all of these references are hereby incorporated herein, in their totalities, by reference).

Those skilled in the art realize that any nucleic acid can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by ribozymes (Draper et al., PCT WO 93/23569, and Sullivan et al., PCT WO 94/02595; Ohkawa et al., 1992, Nucleic Acids Symp. Ser., 27, 15-6; Taira et al., 1991, Nucleic Acids Res., 19, 5125- 30; Ventura et al., 1993, Nucleic Acids Res., 21, 3249-55; Chowrira et al. , 1994, J Biol. Chem., 269, 25856; all of these references are hereby incorporated in their totality by reference herein).

In another aspect of the invention, RNA molecules of the present invention are preferably expressed from transcription units (see, for example, Couture et al., 1996, TIG., 12, 5 10) inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Small interfering RNA expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus.

Preferably, the recombinant vectors capable of expressing the nucleic acid molecules are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of nucleic acid molecules. Such vectors might be repeatedly administered as necessary. Once expressed, the nucleic acid molecule binds to the target mRNA. Delivery of nucleic acid molecule expressing vectors could be by singular, multiple, or chronic delivery by use of the described intracranial access devices.

In one aspect, the invention features an expression vector comprising a nucleic acid sequence encoding at least one functional segment of the nucleic acid molecules of the instant invention. The nucleic acid sequence encoding the nucleic acid molecule of the instant invention is operably linked in a manner which allows expression of that nucleic acid molecule.

In another aspect the invention features an expression vector comprising: a) a transcription initiation region (e.g., eukaryotic pol I, II or III initiation region); b) a nucleic acid sequence encoding at least one of the nucleic acid agents of the instant invention; and c) a transcription termination region (e.g., eukaryotic pol I, II or III termination region);

wherein said sequence is operably linked to said initiation region and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

Transcription of the nucleic acid molecule sequences are driven from a promoter for eukaryotic RNA polymerase I (pol 1), RNA polymerase II (pol II), or RNA polymerase III (pol III) as is known and appreciated in the art. All of these references are incorporated by reference herein. Several investigators have demonstrated that RNA molecules can be expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992, Antisense Res. Dev., 2, 3-15; Ojwang et al., 1992, Proc. NatL Acad Sci. USA, 89, 10802-6; Chen et al., 1992, Nucleic Acids Res., 20, 4581-9; Yu et al., 1993, Proc. Natl. Acad Sci. U S A, 90, 6340-4; L'Huillier et al., 1992, EMBO J, 11, 4411-8; Lisziewicz et al., 1993, Proc. Natl. Acad. Sci. U. S. A, 90, 8000-4; Thompson et al., 1995, Nucleic Acids Res., 23, 2259; Sullenger & Cech, 1993, Science, 262, 1566). More specifically, transcription units such as the ones derived from genes encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as small interfering RNA in cells (Thompson et al., supra; Couture and Stinchcomb, 1996, supra; Noonberg et al., 1994, Nucleic Acid Res., 22, 2830; Noonberg et al., US Patent No. 5,624,803; Good et al., 1997, Gene Ther., 4, 45; Beigelman et al., International PCT Publication No. WO 96118736; all of these publications are incorporated by reference herein). The above small interfering RNA transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, 1996, supra).

It is also important to note that the targeting of ataxin1 mRNA for reduction using a small interfering RNA-based therapy for the disease Spinocerebellar Ataxia Type 1 is but one embodiment of the invention. Other embodiments include the use of an anti-huntingtin small interfering RNA administered to the striatum of the human brain, for the treatment of Huntington's disease, and the use of an anti-alpha-synuclein small interfering RNA administered to the substantia nigra of the human brain, for the treatment of Parkinson's disease.

It should be noted that the exemplified methods for constructing the small interfering RNA to be used as the therapeutic agents in the invention (that is, in vitro transcription from DNA templates and assembly into double-stranded RNA, or cloning the DNA coding for a hairpin structure of RNA into an adeno-associated viral expression vector) are only two possible means for making the therapeutic small interfering RNA. Other larger scale, more efficient methods for manufacturing small interfering RNA may be used to produce the clinical grade and clinical quantities used for treating human patients, without altering the essence of the invention.

Those of skill in the art are familiar with the principles and procedures discussed in widely known and available sources as Remington's Pharmaceutical Science (17th Ed., Mack Publishing Co., Easton, PA, 1985) and Goodman and Gilman's The Pharmaceutical Basis of Therapeutics (8th Ed., Pergamon Press, Elmsford, NY, 1990) both of which are incorporated herein by reference.

In a preferred embodiment of the present invention, the composition comprising the siRNA agent or precursors or or derivatives thereof is formulated in accordance with standard procedure as a pharmaceutical composition adapted for delivered administration to human beings and other mammals. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer.

Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ameliorate any pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

In cases other than intravenous administration, the composition can contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, gel, polymer, or sustained release formulation.

The composition can be formulated with traditional binders and carriers, as would be known in the art. Formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharide, cellulose, magnesium carbonate, etc., inert carriers having well established functionality in the manufacture of pharmaceuticals. Various delivery systems are known and can be used to administer a therapeutic of the present invention including encapsulation in liposomes, microparticles, microcapsules and the like.

In yet another preferred embodiment, therapeutics containing small interfering RNA or precursors or derivatives thereof can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids and the like, and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, thriethylamine, 2-ethylamino ethanol, histidine, procaine or similar.

The amount of the therapeutic of the present invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques, well established in the administration of therapeutics. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and the patient's needs. Suitable dose ranges for intracranial administration are generally about 10^3 to 10^{15} infectious units of viral vector per microliter delivered in 1 to 3000 microliters of single injection volume. Addition amounts of infections units of vector per micro liter would generally contain about 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14} infectious units of viral vector delivered in about 10, 50, 100, 200, 500, 1000, or 2000 microliters. Effective doses may be extrapolated from dose-responsive curves derived from in vitro or in vivo test systems.

For the small interfering RNA vector therapy for neurodegenerative disease of the present invention, multiple catheters having access ports can be implanted in a given patient for a complete therapy. In a preferred embodiment, there is one port and catheter

system per cerebral or cerebellar hemisphere, and perhaps several. Once the implantations are performed by a neurosurgeon, the patient's neurologist can perform a course of therapy consisting of repeated bolus injections of small interfering RNA expression vectors over a period of weeks to months, along with monitoring for therapeutic effect over time. The devices can remain implanted for several months or years for a full course of therapy. After confirmation of therapeutic efficacy, the access ports might optionally be explanted, and the catheters can be sealed and abandoned, or explanted as well. The device material should not interfere with magnetic resonance imaging, and, of course, the small interfering RNA preparations must be compatible with the access port and catheter materials and any surface coatings.

Unless defined otherwise, the scientific and technological terms and nomenclature used herein have the same meaning as commonly understood by a person of ordinary skill to which this invention pertains. Generally, the procedures for cell cultures, infection, molecular biology methods and the like are common methods used in the art. Such standard techniques can be found in reference manuals such as for example Sambrook et al. (1989, Molecular Cloning - A Laboratory Manual, Cold Spring Harbor. Laboratories) and Ausubel et al. (1994, Current Protocols in Molecular Biology, Wiley, New York).

The polymerase chain reaction (PCR) used in the construction of siRNA expression plasmids and/or viral vectors is carried out in accordance with known techniques. See, e.g., U.S. Pat. Nos. 4,683,195; 4,683,202; 4,800,159; and 4,965,188 (the disclosures of all three U.S. Patent are incorporated herein by reference). In general, PCR involves a treatment of a nucleic acid sample (e.g., in the presence of a heat stable DNA polymerase) under hybridizing conditions, with one oligonucleotide primer for each strand of the specific sequence to be detected. An extension product of each primer which is synthesized is complementary to each of the two nucleic acid strands, with the primers sufficiently complementary to each strand of the specific sequence to hybridize therewith. The extension product synthesized from each primer can also serve as a template for further synthesis of extension products using the same primers. Following a sufficient number of rounds of synthesis of extension products, the sample is analyzed to assess whether the sequence or sequences to be detected are present. Detection of the amplified

sequence may be carried out by visualization following EtBr staining of the DNA following gel electrophores, or using a detectable label in accordance with known techniques, and the like. For a review on PCR techniques (see PCR Protocols, A Guide to Methods and Amplifications, Michael et al. Eds, Acad. Press, 1990).

Devices

Using the small interfering RNA vectors previously described, the present invention also provides devices, systems, and methods for delivery of small interfering RNA to target locations of the brain. The envisioned route of delivery is through the use of implanted, indwelling, intraparenchymal catheters that provide a means for injecting small volumes of fluid containing AAV or other vectors directly into local brain tissue. The proximal end of these catheters may be connected to an implanted, intracerebral access port surgically affixed to the patient's cranium, or to an implanted drug pump located in the patient's torso.

Examples of the delivery devices within the scope of the present invention include the Model 8506 investigational device (by Medtronic, Inc. of Minneapolis, MN), which can be implanted subcutaneously on the cranium, and provides an access port through which therapeutic agents may be delivered to the brain. Delivery occurs through a stereotactically implanted polyurethane catheter. The Model 8506 is schematically depicted in Figures 4 and 5. Two models of catheters that can function with the Model 8506 access port include the Model 8770 ventricular catheter by Medtronic, Inc., for delivery to the intracerebral ventricles, which is disclosed in U.S. Patent No. 6,093,180, incorporated herein by reference, and the IPA1 catheter by Medtronic, Inc., for delivery to the brain tissue itself (*i.e.*, intraparenchymal delivery), disclosed in U.S. Serial Nos. 09/540,444 and 09/625,751, which are incorporated herein by reference. The latter catheter has multiple outlets on its distal end to deliver the therapeutic agent to multiple sites along the catheter path. In addition to the aforementioned device, the delivery of the small interfering RNA vectors in accordance with the present invention can be accomplished with a wide variety of devices, including but not limited to U.S. Patent Nos. 5,735,814, 5,814,014, and 6,042,579, all of which are incorporated herein by reference. Using the teachings of the present invention and those of skill in the art will recognize that

these and other devices and systems may be suitable for delivery of small interfering RNA vectors for the treatment of neurodegenerative diseases in accordance with the present invention.

In one preferred embodiment, the method further comprises the steps of implanting a pump outside the brain, the pump coupled to a proximal end of the catheter, and operating the pump to deliver the predetermined dosage of the at least one small interfering RNA or small interfering RNA vector through the discharge portion of the catheter. A further embodiment comprises the further step of periodically refreshing a supply of the at least one small interfering RNA or small interfering RNA vector to the pump outside said brain.

Thus, the present invention includes the delivery of small interfering RNA vectors using an implantable pump and catheter, like that taught in U.S. Patent No. 5,735,814 and 6,042,579, and further using a sensor as part of the infusion system to regulate the amount of small interfering RNA vectors delivered to the brain, like that taught in U.S. Patent No. 5,814,014. Other devices and systems can be used in accordance with the method of the present invention, for example, the devices and systems disclosed in U.S. Serial Nos. 09/872,698 (filed June 1, 2001) and 09/864,646 (filed May 23, 2001), which are incorporated herein by reference.

To summarize, the present invention provides methods to deliver small interfering RNA vectors to the human central nervous system, and thus treat neurodegenerative diseases by reducing the production of a pathogenic protein within neurons.

The present invention is directed for use as a treatment for neurodegenerative disorders and/or diseases, comprising Alzheimer's disease, Parkinson's disease, Huntington's disease, Spinocerebellar type 1, type 2, and type 3, and/or any neurodegenerative disease caused or aggravated by the production of a pathogenic protein, or any other neurogenerative disease caused by the gain of a new, pathogenic function by a mutant protein.

Examples

Example 1: Construction of a small interfering RNA targeting human ataxin1 mRNA.

As an example of the embodiments of the invention, we have made a small interfering RNA that targets the mRNA for human ataxin1. This small interfering RNA reduces the amount of mRNA for human ataxin1 in human cells, in cell cultures. As a therapy for Spinocerebellar Ataxia Type 1 (SCA1), this same small interfering RNA or a similar small interfering RNA will be delivered to the cells of the cerebellum in the patient's brain, using implanted access ports and catheters. The result will be a reduction in the amount of ataxin1 protein in these cells, thereby slowing or arresting the progression of the patient's SCA1 disease.

The small interfering RNA against human ataxin1 was been constructed from the nucleotide sequence for human ataxin1. The sequence from human ataxin 1 was retrieved from the publicly-accessible nucleotide database provided by NCBI, retrievable as NCBI accession number NM_000332 (SEQ ID:15). A portion of the human mRNA sequence for ataxin1 was identified as a potential site for small interfering RNA cleavage and also predicted to be single-stranded by MFOLD analysis. In accession NM_000332 (SEQ ID:15), three pairs of anti ataxin1 siRNA targets were constructed:

1. Anti-ataxin1 siRNA targeting the mRNA sequence at sites numbered 945 through 965:

SEQ ID:1 5' - AACCAAGAGCGGAGCAACGAA - 3'
SEQ ID:2 3' - GGTTCTCGCCTCGTTGCTTAA - 5'

2. Anti-ataxin1 siRNA targeting the mRNA sequence at sites numbered 1671 - through 1691:

SEQ ID:3 5' - AACCAAGAGCGGAGCAACGAA - 3'
SEQ ID:4 3' - GGTTCTCGCCTCGTTGCTTAA - 5'

3. Anti-ataxin1 siRNA targeting the mRNA sequence at sites numbered 2750 - through 2770:

SEQ ID:4 5' - AACCAGTACGTCCACATTTCC - 3'
SEQ ID:6 3' - GGTCATGCAGGTGTAAAGGAA - 5'

A series of six deoxyoligonucleotide fragments were designed, ordered and purchased from the MWG Biotech, Inc., custom oligonucleotide synthesis service to provide the six fragments making up the three target sites. Additionally, these oligonucletides were constructed to include an 8 base sequence complementary to the 5' end of the T7 promoter primer included in an siRNA construction kit (Ambion, Inc. catalog number 1620). Each specific oligonucleotide was annealed to the supplied T7 promoter primer, and filled-in with Klenow fragment to generate a full-length DNA template for transcription into RNA. Two in vitro transcribed RNAs (one athe antisense to the other) were generated by in vitro transcription reactions then hybridized to each other to make double-stranded RNA. The double-stranded RNA product was treated with DNase (to remove the DNA transcription templates) and RNase (to polish the ends of the double-stranded RNA), and column purified to provide the three siRNAs that were delivered and tested in cells.

Example 2: Delivery of a small interfering RNA targeting human ataxin1 mRNA.

The constructed siRNA molecules 1-3 described in Example 1 were transfected into HEK293 cells. The RNA produced by the transfected cells was harvested and assayed to measure the amount of human ataxin1 mRNA.

Figure 1 shows the results of a quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) assay for the amount of ataxin1 messenger RNA (mRNA) per microgram of total RNA from cultures of HEK 293H cells. Four cell populations were

assayed. The first were 293H cells that had been transiently transfected with siRNA against GAPDH, a "housekeeping gene" with no known relationship to ataxin1 mRNA expression. (The siRNA against GAPDH was supplied as a standard control by Ambion, Inc., in their commercially-available kit for making and testing siRNA). The second were 293H cells that had been transiently transfected with siRNA against ataxin1 mRNA at location 1671 in the ataxin1 mRNA sequence. The third were 293H cells transiently transfected with a plasmid containing a ribozyme against ataxin1 mRNA (which cleaves ataxin1 mRNA at position 1364 in the ataxin1 mRNA sequence). The fourth were 293H cells transiently transfected with siRNA against ataxin1 mRNA at location 0945. All cell populations were harvested concurrently for total cellular RNA, at a time point 48 hours after transfection.

On the gels pictured, the amplified DNA products of the RT-PCR reaction were separated by molecular size, using gel electrophoresis, and are visible as bands of varying intensity. Each cell population described was assayed using a series of parallel reactions, shown as a set of lanes at the top or bottom of each gel. Each set of lanes contains two bands per lane. The top band is the DNA product amplified from a known quantity of DNA added to the reaction to compete with the endogenous cDNA reverse transcribed from the cellular mRNA. If the bands in a given lane are of the same intensity, then the amount of cellular mRNA in the original cell sample can be inferred to be equivalent to the amount of known quantity of DNA added to the reaction tube. From left to right across the lanes, the amount of known DNA standard added was decreased, in the picogram amounts shown. The assay is interpreted by looking for the set of lanes for which the intensity of the bands "crosses over" from being brightest for the DNA standard, to being brightest for the cellular product below it, indicating that the amount of DNA standard is now lower than the amount of cellular mRNA.

On the gel shown in Figure 1, the top set of lanes is from the cells transfected with the ribozyme against ataxin1 mRNA. The comparison of the bands from this cellular sample to the bands from the DNA standards indicates that the amount of ataxin1 mRNA in these cells is between .505 and .303 picograms per microgram of total cellular RNA. The bottom set of lanes is from the cells transfected with siRNA against ataxin1 at

position 0945. Analysis of these lanes indicates that the amount of ataxin1 mRNA in these cells is between .303 and .202 picograms per microgram of total cellular RNA.

On the gelshown in Figure 2, the top set of lanes is from the cells transfected with a control siRNA against GAPDH. Analysis of these lanes indicates that the amount of ataxin1 mRNA in these cells is between .711 and .400 picograms per microgram of total cellular RNA. Finally, the bottom set of lanes is from cells transfected with another siRNA against ataxin1, at position 1671. These lanes indicate that the amount of ataxin1 mRNA in these cells is between 0.404 and 0.303 picograms per microgram of total cellular RNA.

In summary, the results of this particular analysis were:

Treatment	Amount of ataxin1 mRNA (picograms per microgram total cellular RNA)		
	Lower bound	Upper bound	Midpoint Estimate
Control (GAPDH)	0.400	0.711	0.555
Ribozyme (A1364A)	0.303	0.505	0.404
siRNA (AT1671)	0.303	0.404	0.353
siRNA (AT0945)	0.202	0.303	0.252

These data indicate that both the AT1671 and AT0945 siRNA against ataxin1 were effective at reducing the amount of ataxin1 mRNA in these cells within 48 hours after transfection, and that the siRNA were more effective at the reduction of ataxin1 mRNA than was this anti-ataxin1 ribozyme.

It should be noted that the exemplified method for constructing the small interfering RNA to be used as the therapeutic agents in the invention (that is, assembly from oligonucleotides using in vitro transcription and hybridization) is only one possible means for making the therapeutic small interfering RNA. Other larger scale, more efficient methods for manufacturing small interfering RNA may be used to produce the clinical grade and clinical quantities used for treating human patients, without altering the essence of the invention or departing from the spirit and scope of this invention, as set

forth in the appended claims.

Example 3: Allele-Specific Reduction of Ataxin1 Expression Using Small, Interfering RNA

In heterozygous patients, if a single nucleotide polymorphism (SNP) were to differ between the mutant and normal length allele, an appropriate siRNA might selectively reduce expression of only the mutant allele. We have tested 293, DAOY, SK-N-SH, and HeLa cells using allele-specific RT-PCR for a SNP at position +927 downstream from the SCA1 start codon (see Accession NT_007592). HeLa cells express a 927C but no 927T allele, while 293 cells express a 927T but no 927C allele. DAOY and SK-N-SH cells express both allelic variants. We have created allele-specific siRNA centered at this site. Results of assays for allele-specific suppression of endogenous SCA1 mRNA by these siRNA variants will be presented.

Example 4: Construction of Small, Interfering RNA Viral Vectors

A selectable reporter plasmid, pAAV-U6-Tracer is constructed for cloning siRNA. (See Figure 3). The plasmid pAAV-U6-Tracer is constructed to contain the inverted terminal repeats (ITR) of adeno-associated virus, flanking the U6 RNA polymerase III promoter from pSilencer (Ambion), and the EF1a promoter, green fluorescence protein, Zeocin^r resistance, and SV40 poly A from pTracer (Invitrogen). The gene segments are cloned as shown in Figure 3. Oligonucleotides for expressing siRNA are cloned into the multiple cloning region just downstream in the 3' direction from the U6 RNA polymerase III promoter.

HEK293 Cells are cotransfected with pAAV-siRNA, pHelper, and pAAV-RC to make viral producer cells, where the pAAV-RC and pHelper plasmids are part of the three plasmid AAV production system Avigen, Inc.) . The producer 293 cells are grown in culture are used to isolate recombinant viruses, which is used to transfect secondary cells: HeLa Cells, DAOY cells, and SK-N-SH cells.

WE CLAIM:

1. A medical system for treating a neurodegenerative disorder comprising:
   a. an intracranial access device;
   b. a mapping means for locating a predetermined location in the brain;
   c. a deliverable amount of a small interfering RNA or vector encoding said small interfering RNA; and
   d. a delivery means for delivering said small interfering RNA or vector encoding said small interfering RNA to said location of the brain from said intracranial access device.

2. A medical system of claim 1 wherein said neurodegenerative disorder is Parkinson's disease.
3. A medical system of claim 1 wherein said neurodegenerative disorder is Alzheimer's disease.
4. A medical system of claim 1 wherein said neurodegenerative disorder is Huntington's disease.
5. A medical system of claim 1 wherein said neurodegenerative disorder is spinocerebellar ataxia type 1.
6. A medical system of claim 1 wherein said neurodegenerative disorder is spinocerebellar ataxia type 2.
7. A medical system of claim 1 wherein said neurodegenerative disorder is spinocerebellar ataxia type 3, also known as Machado-Joseph disease.
8. A medical system of claim 1 wherein said neurodegenerative disorder is dentatorubral-pallidoluysian atrophy, also known as DRPLA.
9. A medical system of claim 1 wherein said intracranial access device is an intracranial catheter.
10. A medical system of claim 1 wherein said intracranial access device is an intracranial access port.

11. A medical system of claim 1 wherein said predetermined location is the substantia nigra.

12. A medical system of claim 1 wherein said predetermined location is the nucleus basalis of Meynert or the cerebral cortex.

13. A medical system of claim 1 wherein said predetermined location is the caudate nucleus, the putamen, or the striatum.

14. A medical system of claim 1 wherein said predetermined location is the dentate nucleus, emboliform nucleus, the globose nucleus, the fastigial nucleus of the cerebellum (collectively the deep cerebellar nuclei), or the cerebellar cortex.

15. A medical system of claim 1 wherein said predetermined location is the subthalamic nucleus.

16. A medical system of claim 1 wherein said small interfering RNA is complementary to the mRNA for alpha-synuclein.

17. A medical system of claim 1 wherein said small interfering RNA is complementary to the mRNA for beta amyloid cleaving enzyme type 1, or BACE1.

18. A medical system of claim 1 wherein said small interfering RNA is complementary to the mRNA transcript from the IT15 gene, including the code for the huntingtin protein.

19. A medical system of claim 1 wherein said small interfering RNA is complementary to the mRNA transcript from the SCA1 gene, including the code for the ataxin1 protein.

20. A medical system of claim 1 wherein said small interfering RNA is complementary to the mRNA transcript from the SCA2 gene, including the code for the ataxin2 protein.

21. A medical system of claim 1 wherein said small interfering RNA is complementary to the mRNA transcript from the SCA3 gene, including the code for the ataxin3 protein, also known as the Machado-Joseph protein.

22. A medical system of claim 1 wherein said small interfering RNA is complementary to the mRNA transcript from the DRLPA gene, including the code for the atrophin1 protein.

23. A medical system of claim 1 wherein said small interfering RNA is substantially provided for in any one of **SEQ ID Nos: 1-44.**

24. A medical system of claim 1 wherein said delivery means is injection from an external syringe into an intracranial access port.

25. A medical system of claim 1 wherein said delivery means is an infusion pump.

26. An infusion pump of claim 25 wherein the said infusion pump is an electromechanical pump.

27. An infusion pump of claim 25 wherein the said infusion pump is an osmotic pump.

28. A method for treating a neurodegenerative disorder comprised of modulating the expression or production of a protein in neurons by intracranial delivery of a small interfering RNA that reduces said expression of production of said protein, in a pharmaceutically acceptable carrier.

29. A method of delivering a small interfering RNA to a location in the brain comprising the steps of:

    a. surgically implanting an intracranial access delivery device; and

    b. infusing a small interfering RNA and/or a vector encoding said small interfering RNA at a predetermined site in the brain.

30. A method of delivering a small interfering RNA to a location in the brain comprising the steps of:

    a. surgically implanting an intracranial access delivery device; and

    b. infusing a small interfering RNA and/or a vector encoding said small interfering RNA at a predetermined site in the brain; wherein at least one attribute of said neurodegenerative diseases is reduced or its progression slowed or arrested.

31. The method of claim 30, wherein said step of implanting the catheter is performed after said neurodegenerative disorder is diagnosed.

32. The method of claim 31, wherein said step of implanting the catheter is performed after said neurodegenerative disorder is diagnosed and before the symptoms of the said neurodegenerative disorder are manifest.

33. The method of claim 31, wherein said step of implanting the catheter is performed after said neurodegenerative disorder is diagnosed and after the symptoms of the said neurodegenerative disorder are manifest.

34. The method of any one of claims 29, 30, or 31, wherein said intracranial access delivery device is an intracranial access port coupled to the proximal end of an intracranial catheter.

35. The method of any one of claims 29, 30, or 31, further comprising the steps of: implanting a pump outside the brain, the pump coupled to the proximal end of an intracranial catheter.

36. The method of claim 35 comprising operating the pump to deliver a predetermined dosage of the said small interfering RNA or vector encoding said small interfering RNA from the pump through the discharge portion of the said intracranial catheter.

37. The method of claim 35 further comprising the step of periodically refreshing the pump with at least one substance.

38. The method of claim 35 wherein said pump is an infusion pump.

39. The method of claim 38 wherein said infusion pump is an electromechanical pump.

40. The method of claim 38 wherein said infusion pump is an osmotic pump.

41. A method of claims 28 or 30, wherein said neurodegenerative disorder is Parkinson's disease.

42. A method of claims 28 or 30 wherein said neurodegenerative disorder is Alzheimer's disease.

43. A method of claims 28 or 30, wherein said neurodegenerative disorder is Huntington's disease.

44. A method of claims 28, or 30 wherein said neurodegenerative disorder is spinocerebellar ataxia type 1.

45. A method of claims 28 or 30, wherein said neurodegenerative disorder is spinocerebellar ataxia type 2.

46. A method of claims 28 or 30, wherein said neurodegenerative disorder is spinocerebellar ataxia type 3, also known as Machado-Joseph disease.

47. A method of claims 28 or 30, wherein said neurodegenerative disorder is dentatorubral-pallidoluysian atrophy, also known as DRPLA.

48. A method of claims 29 or 30, wherein the said predetermined site in the brain is the substantia nigra.

49. A method of claims 29 or 30, wherein the said predetermined site in the brain is the nucleus basalis of Meynert or the cerebral cortex.

50. A method of claims 29 or 30, wherein the said predetermined site in the brain is the caudate nucleus, the putamen, or the striatum.

51. A method of claims 29 or 30, wherein the said predetermined site in the brain is the dentate nucleus, emboliform nucleus, the globose nucleus, the fastigial nucleus of the cerebellum (collectively the deep cerebellar nuclei), or the cerebellar cortex.

52. A method of claims 29 or 30, wherein the said predetermined site in the brain is the subthalamic nucleus.

53. A method of claims 28, 29, or 30, wherein said small interfering RNA is complementary to the mRNA for alpha-synuclein.

54. A method of claims 28, 29, or 30 wherein said small interfering RNA is complementary to the mRNA for beta amyloid cleaving enzyme type 1, or BACE1.

55. A method of claims 28, 29 or 30 wherein said small interfering RNA is complementary to the mRNA transcript from the IT15 gene, including the code for the huntingtin protein.

56. A method of claims 28, 29, or 30 wherein said small interfering RNA is complementary to the mRNA transcript from the SCA1 gene, including the code for the ataxin1 protein.

57. A method of claims 28, 29, or 30 wherein said small interfering RNA is complementary to the mRNA transcript from the SCA2 gene, including the code for the ataxin2 protein.

58. A method of claims 28, 29, or 30 wherein said small interfering RNA is complementary to the mRNA transcript from the SCA3 gene, including the code for the ataxin3 protein, also known as the Machado-Joseph protein.

59. A method of claims 28, 29 or 30 wherein said small interfering RNA is complementary to the mRNA transcript from the DRLPA gene, including the code for the atrophin1 protein.

60. A method of claims 28, 29, or 30 wherein said small interfering RNA is delivered by a delivery vector.

61. A method of claim 60 wherein the delivery vector is adeno-associated virus, or AAV.
62. A method of claim 60 wherein the delivery vector is adenovirus.
63. A method of claim 60 wherein the delivery vector is herpes simplex virus, or HSV.
64. A method of claim 60 wherein the delivery vector is lentivirus.
65. A method of claim 60 wherein the delivery vector is a DNA plasmid.
66. A method of claim 65 wherein the said DNA plasmid is complexed with a liposomal compound.
67. A method of claim 65 wherein the said DNA plasmid is complexed with polyethylenimine (PEI).
68. A small interfering RNA containing sequences according to **SEQ ID Nos 1-4-**, or a partial sequence thereof, or a base sequence hybridizable to a complementary strand of RNA encoding a protein associated with a neurodegenerative disease.
69. A small interfering RNA comprising an RNA sequence hybridizable to the RNA sequence encoding a protein associated with a neurodegenerative disease to cause cleavage of said protein-encoding RNA sequence.
70. A small interfering RNA expression sequence comprising the DNA sequence encoding an RNA sequence hybridizable to the RNA sequence encoding a protein associated with a neurodegenerative disease to cause cleavage of said protein-encoding RNA sequence.
71. A small interfering RNA of any of claims 68, 69, or 70 wherein said neurodegenerative disease is Parkinson's disease.
72. A small interfering RNA of any of claims 68, 69, or 70 wherein said neurodegenerative disease is Alzheimer's disease.
73. A small interfering RNA of any of claims 68, 69, or 70 wherein said neurodegenerative disease is Huntington's disease.
74. A small interfering RNA of any of claims 68, 69, or 70 wherein said neurodegenerative disease is spinocerebellar ataxia type 1.
75. A small interfering RNA of any of claims 68, 69, or 70 wherein said neurodegenerative disease is spinocerebellar ataxia type 2.

76. A small interfering RNA of any of claims 68, 69, or 70 wherein said neurodegenerative disease is spinocerebellar ataxia type 3, also known as Machado-Joseph disease.

77. A small interfering RNA of any of claims 68, 69, or 70 wherein said neurodegenerative is dentatorubral-pallidoluysian atrophy, also known as DRPLA.

78. A small interfering RNA of any of claims 68, 69, or 70 wherein said small interfering RNA is complementary to the mRNA for alpha-synuclein.

79. A small interfering RNA of any of claims 68, 69, or 70 wherein said small interfering RNA is complementary to the mRNA for beta amyloid cleaving enzyme type 1, or BACE1.

80. A small interfering RNA of any of claims 68, 69, or 70 wherein said small interfering RNA is complementary to the mRNA transcript from the IT15 gene, including the code for the huntingtin protein.

81. A small interfering RNA of any of claims 68, 69, or 70 wherein said small interfering RNA is complementary to the mRNA transcript from the SCA1 gene, including the code for the ataxin1 protein.

82. A small interfering RNA of any of claims 68, 69, or 70 wherein said small interfering RNA is complementary to the mRNA transcript from the SCA2 gene, including the code for the ataxin2 protein.

83. A small interfering RNA of any of claims 68, 69, or 70 wherein said small interfering RNA is complementary to the mRNA transcript from the SCA3 gene, including the code for the ataxin3 protein, also known as the Machado-Joseph protein.

84. A small interfering RNA of any of claims 68, 69, or 70 wherein said small interfering RNA is complementary to the mRNA transcript from the DRLPA gene, including the code for the atrophin1 protein.

293H Cells Transfected with Anti-Ataxin1 Ribozyme (A1364A) and Anti-ataxin siRNA (AT0945)

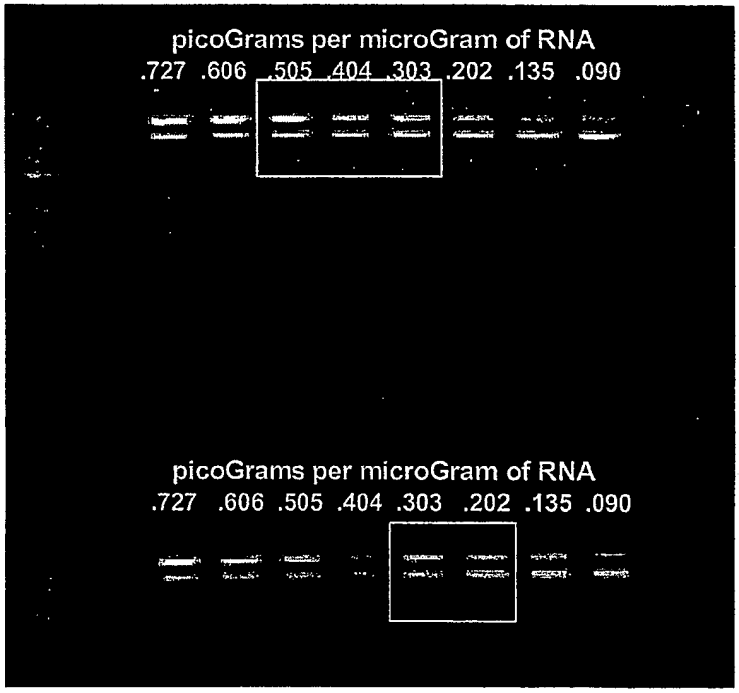

FIG. 1

293H Cells Transfected with Control siRNA (GAPDH) and Anti-ataxin siRNA (AT1671)

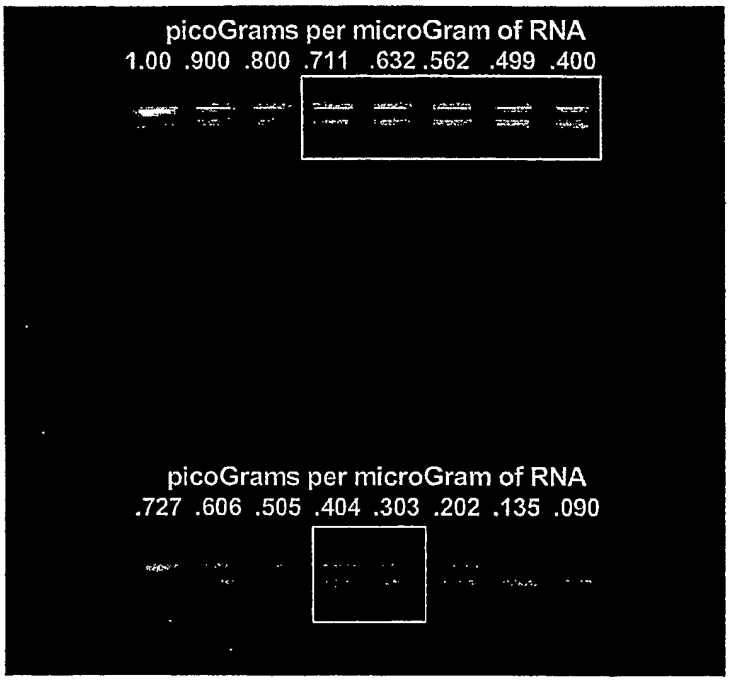

Fig. 2

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pAAV-MCS
ITR
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EF1a promoter
GFP/Zeocin'
SV40 polyA
ITR
EcoRV
pAAV-Tracer
ITR
PCR primer
U6 pol III
pSilencer
PCR
ClaI
EcoRV
EcoRI
ClaI
EcoRV
EcoRI
pAAV-U6-Tracer
ITR
GFP-
Zeocin'
ITR
DNA coding for siRNA expression
Sense Loop AS U6term
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Fig. 3

62

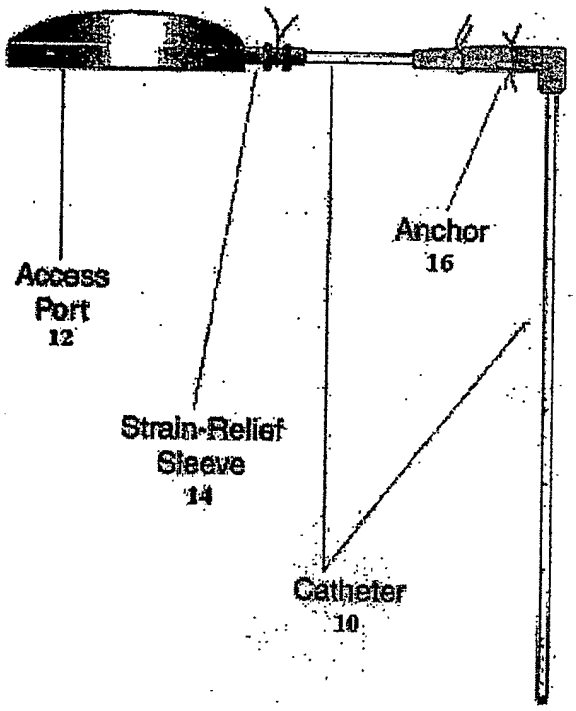
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Figure. 4

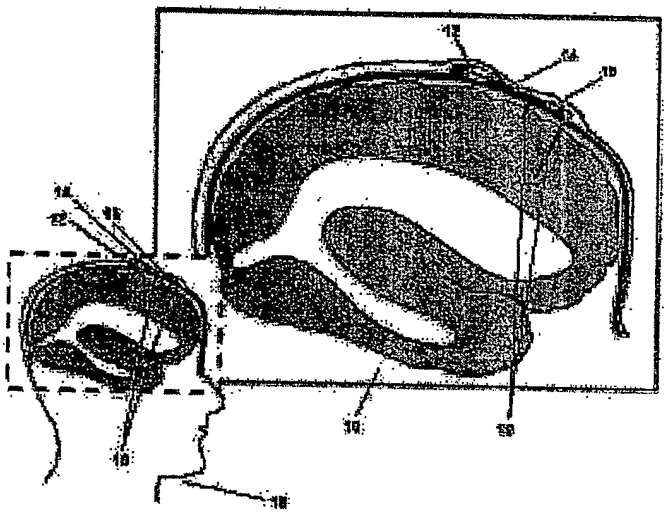

Fig. 5

Small interfering RNA Treatment of Neurodegenerative Diseases

Disease	Location	Gene Product
Parkinson's Disease	Substantia Nigra	alpha-synuclein
Alzheimer's Disease	Nucleus Basalis of Meynert Cerebral Cortex	BACE1 (including variants thereof, e.g. variants A, B, C, and D)
Huntington's Disease	Striatum: Caudate Nucleus Putamen	Huntingtin (i.e., the protein product of the Huntington's gene IT15)
Spinocerbellar Ataxia Type 1 Type 2 Type 3 (Machado Joseph)	Deep Cerebellar Nuclei: Dentate nucleus Emboliform nucleus Globose nucleus Fastigial nucleus Cerebellar cortex	Ataxin 1 Ataxin 2 Ataxin 3
Dentatorubral-pallidoluysian atrophy	Red Nucleus Globus Pallidus	Atrophin 1

Fig. 6

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axin 3) (MJD) . . .
ACCESSION NM_030660

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